bio-protocol Preprint

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Neutrophil conditioned media collection and inhibition.

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An abbreviated version of this protocol was published in Science Translational Medicine in Oct 2021

Skeletal muscle regeneration with robotic actuation–mediated clearance of neutrophils

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Detailed protocol

1. Isolate neutrophils from 6–8-week-old female C57BL6/J mice.
    - 1 μg/ml of Granulocyte-macrophage colony-stimulating factor (GM-CSF, Peprotech) loaded in 5 mg ofmesoporous silica rods suspended in PBS was subcutaneously injected into the left flank of C57BL6/J mice
    - After 3 to 6 days, neutrophils are isolated from MSRs, peripheral blood, and spleen using a mouse neutrophil isolation kit (Miltenyi Biotec)
2. Freshly isolated neutrophils are incubated with 100 nM ofPhorbol 12-myristate 13-acetate (PMA) in Hanks' balanced salt solution (HBSS) with 0.25% BSA for 2 hours for activation.
3. Neutrophils were then thoroughly washed with HBSS and cultured at the density of 1 million cells/ml in RPMI supplemented with 1% heat-inactivated FBS and 1% P/S for 12 hours.
4. The conditioned media, containing neutrophil-derived factors, was collected and used after centrifugation to remove dead cells and debris.
5. Neutrophil-free RPMI supplemented with 1% heat-inactivated FBS and 1% P/S was incubated at 37°C for 12 hours and used as control media for NeutCM.
6. To block functionality of specific factors in NeutCM, NeutCM was incubated with neutralizing antibodies [100 ng/ml of anti-Cxcl2 (Novus Biologicals), 100 ng/ml of anti-Ccl3 (Novus Biologicals), 100 ng/ml of anti-Lipocalin2 (Novus Biologicals), 1 μg/ml of anti Mmp-9 (Invitrogen)] and their IgG controls overnight at 4°C and then added to cell culture media.

This protocol is included in our published work (Seo et al., Sci Transl Med, 2021)

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Seo, B. and Mooney, D. (2021). Neutrophil conditioned media collection and inhibition.. Bio-protocol Preprint. bio-protocol.org/prep1452.
2. Seo, B. R., Payne, C. J., McNamara, S. L., Freedman, B. R., Kwee, B. J., Nam, S., Lázaro, I. D., Darnell, M., Alvarez, J. T., Dellacherie, M. O., Vandenburgh, H. H., Walsh, C. J. and Mooney, D. J.(2021). Skeletal muscle regeneration with robotic actuation–mediated clearance of neutrophils. Science Translational Medicine 13(614). DOI: 10.1126/scitranslmed.abe8868